Age related metabolic consequences of reduced myelin basic protein – MRS and MRI of heterozygous Shiverer mice

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PURPOSE: Several studies performed post mortem on brains of patients with schizophrenia, bipolar disorder and depression revealed a reduced expression of about 30-40% of the myelin basic protein (MBP), a major myelin structural protein. However, causes and consequences of this reduction are entirely unknown. Heterozygous Shiverer mice (Shiv+/-) express similarly reduced MBP levels, but compared to MBP null mutants (Shiv-/-) these mice exhibit only very mild myelin alterations in histology and did not show the typical shivering phenotype[1]. Thus, these mice may be a suitable model to resemble at least parts of the human situation. The purpose of this study was to analyze firstly whether this mild myelin alteration can be detected by MRI including magnetization transfer techniques and diffusion tensor imaging, secondly whether these mild changes in MBP level have metabolic consequences as it has been reported for Shiv-/- and mental disorders in humans as well, and thirdly how this phenotype may develop with age with the overall aim to contribute to an better understanding of the role of myelin proteins in development of mental disease.

METHODS: 24 adult (6 months: 12 Shiv+/- and 12 wild types [WT]) and 32 elderly (>18 months: 16 Shiv+/- and 16 WT) male mice were examined in accordance with German animal protection laws after approval by the responsible governmental authority. The mice were anaesthetized by isoflurane and actively ventilated via endotracheal tube. The body temperature was kept constant at 36.0±0.5°C. MRI and MRS measurements were employed at 7 T (ClinScan, Bruker BioSpin) and comprised T2-weighted images (2D FSE, TR/TE=4000/50 ms), diffusion-weighted images (2D EPI, TR/TE= 7000/28 ms, 12 directions, b= 0/1000 s/mm²), and 3 differently weighted 3D FLASH based datasets (TR/TE=28/1.9 ms, flip angle=25° for T1- and 5° for proton weighting, the later with and without additional MT-weighting by Gaussian shaped off resonance pules). Maps of MTR, MT saturation (MT sat) corrected for signal amplitude and T1 (T1app)[2], FA, ADC, axial-diffusivity- and radial-diffusivity were calculated using in house Matlab scripts (Mathworks Natick, USA). Localized proton MR spectra (PRESS, TR/TE= 6000/10 ms) were obtained from VOIs in the hippocampus (1.8x0.7x1.8 mm^3), cortex (3.9x0.7x3.2 mm^3) and corpus callosum (3.9x0.7x1.7mm^3 CC). Metabolite quantification involved spectral evaluation by LCModel (Version 6.3-0G, Provencher, 1993). Results with Cramer-Rao lower bounds above 20% were excluded from further analyses. Finally mice were sacrificed and prepared for histology.

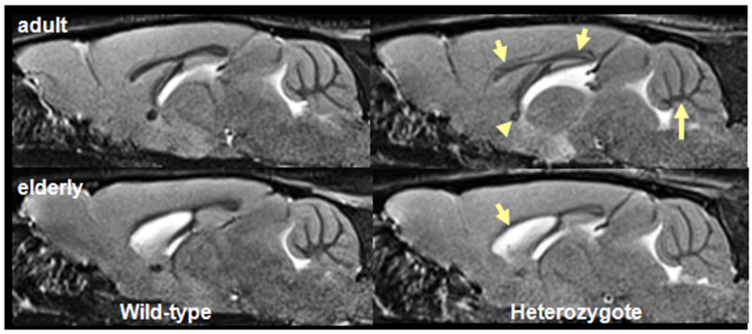

Fig. 1: T2 weighted images demonstrate a subtle reduction o gray-white matter contrast and minor thinning of the cc which was more severe in elderly animals

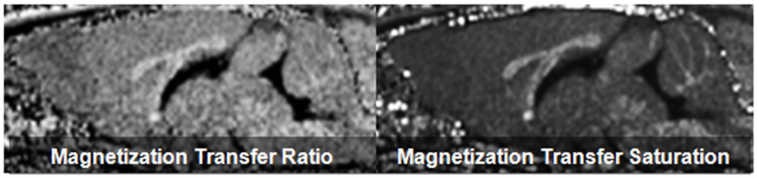

Fig. 2: MT_{Sat} reveals a superior contrast compared to the MTR

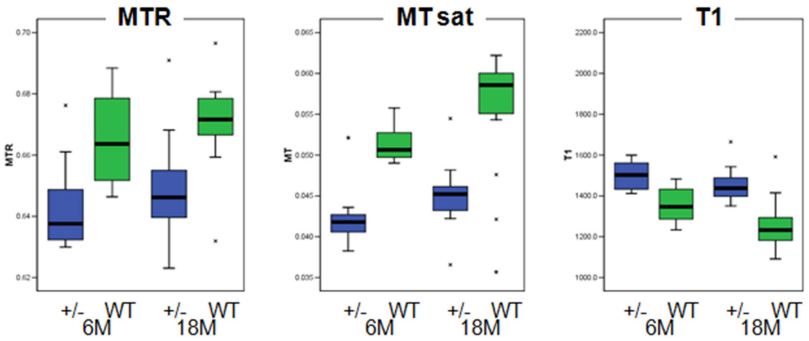

Fig. 3: ROI analyses of the cc reveal significant differences between Shiv+/- and WT. Elderly animals were comparable to adult animals.

RESULTS: Visual inspection of the T2-weighted images (Fig. 1) demonstrated a subtle reduction of gray-white matter contrast and a mild thinning of the CC in Shiv+/-. In the same line, ROI analysis revealed a significant reduction of MTR and MT sat and a significant increase of T1 in the CC of Shiv+/- compared to wild type mice which became particularly evident with high age. In contrast, no significant changes of the diffusion derived parameter were observed. Moreover, the CC of Shiv+/- showed an increase of myo-inositol and taurine, the latter become only prominent with age. Likewise only apparent in the elderly mice was an increase of total creatine in the cortex. No changes of N-acetyl aspartate or choline were observed.

CONCLUSION: The subtle myelin alterations in Shiv+/- mice were best detectable by MT saturation. The lack of changes in radial diffusivity might be due to the nature of the myelin alteration mainly characterized by abnormal myelin wrapping. In addition mild microglia activation (increase in myo-inositol) might also compromise the DTI results. Changes in total creatine and taurine may indicate altered energy metabolism and have been described in patients with mental illness[3]. These finding further underline the requirement of absolute metabolite quantification instead of using ratios normalized to total creatine. The observed phenotype became more evident with age. Based on the present findings together with the observed behavioral changes, we conclude that the MBP reduction in mental illness may indeed be responsible for part of the typical disease phenotypes based on disturbed connectivity.

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